

STIC-ILL

From: Baskar, Padmavathi
Sent: Monday, June 23, 2003 12:33 PM
To: STIC-ILL
Subject: 09/300612, please consider this as rush

Bone "The Pathogenesis of Sepsis" Ann Int Med 115(6) 457-469 1991.

Guyton "Textbook of Medical Physiology" 8th Ed 269-271 1991.

Mousstach e et al "Mechanisms of Resistance of the Opossum to Some Snake Venoms" Toxicon 17(Suppl. 1) 130 1979.

Domont et al. "Natural Anti-Snake Venom Proteins" Toxicon 29(10) 1183-1194 1991.

Perales et al "Neutralization of the Oedematogenic activity of Bothrops Jararaca venom on the Mouse Paw by an antithroptic

Fraction Isolated from Opossum serum" Agents Actions 37(3-4) 250-259 1992.

Tomihara et al. "Purification of Three Antihemorrhagic Factors From The Serum of A Mongoose" Toxicon 25(6) 685-689 1987.

Perates et al. "Anti Snake Venom Protein from Didelphidae" Abstract 10th World Congress. Toxicon 30(5-6) 543 1992.

Menchaca et al. "The Purification & Characterization of An Antihemorrhagic Factor in Opossum Serum" Toxicon 19(5) 623-632 1981.

Toxicon 14(4) 337-340, 1976

Tarng et al , Toxicon 24(6) 567-573, 1986.

Toxicon 34(11-12) 1313-6, 1996.

Toxicon 36(10) 1451-9, 1998.

HIGH TECH SEPARATIONS NEWS 1996, V9,N4, SEP1996

Toxicon 37(6) 949-954, 1999.

Toxicon 37(5) 703-728, 1999.

biochimica et biophysica acta 1995, 1245 (2) 232-8

Padma Baskar
Art Unit 1645
Patent Examiner/Biotechnology
CM-1, 8E-13
703-308-8886

The Pathogenesis of Sepsis

Roger C. Bone, MD

■ Sepsis and its sequelae (sepsis syndrome and septic shock) are increasingly common and are still potentially lethal diagnoses. Many mediators of the pathogenesis of sepsis have recently been described. These include tumor necrosis factor α (TNF α), interleukins, platelet activating factor, leukotrienes, thromboxane A₂, and activators of the complement cascade. Neutrophil and platelet activation may also play a role. Other agents that may participate in the sepsis cascade include adhesion molecules, kinins, thrombin, myocardial depressant substance, β -endorphin, and heat shock proteins. Endothelium-derived relaxing factor and endothelin-1 are released from the endothelium and seem to exert a regulatory effect, counterbalancing each other.

A central mediator of sepsis does not seem to exist, although TNF α has been commonly proposed for this role. Animal studies are difficult to extrapolate to the clinical setting because of cross-species differences and variations in experimental design. Rather than being caused by any single pathogenic mechanism, it is more likely that sepsis is related to the state of activation of the target cell, the nearby presence of other mediators, and the ability of the target cell to release other mediators. Also important is the downregulation or negative feedback of these mediators or the generation of natural inflammation inhibitors, such as interleukin-4 and interleukin-8.

Endothelial damage in sepsis probably results from persistent and repetitive inflammatory insults. Eventually, these insults produce sufficient damage that downregulation can no longer occur; this leads to a state of metabolic anarchy in which the body can no longer control its own inflammatory response.

Advances in medical practice and technology have increased the risk of sepsis and its sequelae (the sepsis syndrome and septic shock) (1-3). Among these are the aggressive use of catheters and other invasive equipment; implantation of prosthetic devices; and administration of chemotherapy to cancer patients or of corticosteroids and other immunosuppressive agents to patients with organ transplants or inflammatory diseases. In addition, improvements in medical care have given longer life spans to the elderly and to patients with metabolic, neoplastic, or immunodeficiency disorders—but these groups remain at increased risk for infection.

Hope is in sight, however. Discoveries made in the last few years have vastly increased our understanding of the pathogenesis of sepsis and its sequelae. Many of the mediators responsible for the endothelial derangements underlying these disorders have been identified. Even more important, these discoveries have led to a number of new therapies—treatments that hold promise of significantly reducing the high mortality associated with sepsis. Many of these new therapies are now under investigation and may soon be available for clinical use.

My purpose here is to summarize what we have learned about these new mediators and then to propose a new hypothesis for understanding how these mediators produce the endothelial dysfunction that is widely believed to be one of the key derangements underlying sepsis and its sequelae. In brief, I submit that these disorders result from panendothelial injury, caused by repetitive, localized foci of inflammation, which, in turn, produces an increase in capillary permeability. I do not mean to imply that endothelial damage is the sole cause of these disorders. Metabolic derangements, coagulative disorders, and myocardial depression undoubtedly also play a role. Only by recognizing that patients with sepsis, the sepsis syndrome, or septic shock have multiple local defects in endothelial permeability, however, can we begin to formulate effective treatment regimens.

Epidemiology

It is difficult to establish the true incidence of sepsis and its sequelae for two reasons. First, there has been a lack of consensus about the definition of these diseases. This problem is perhaps best illustrated by the fact that reports of mortality associated with septic shock range from 10% (4) to 90% (1). To overcome this problem, a framework for defining levels of infection from uncomplicated bacteremia through refractory septic shock has recently been proposed (5) and is presented in Table 1.

The second problem is that none of the disorders listed in Table 1 is a reportable disease. However, recent data from the Centers for Disease Control suggest

Annals of Internal Medicine. 1991;115:457-469.

From Rush Medical College, Chicago, Illinois. For the current author address, see end of text.

Table 1. A Uniform System for Defining the Spectrum of Disorders Associated with Sepsis*

Disorder	Requirements for Clinical Diagnosis
Bacteremia†	Positive blood cultures
Sepsis	Clinical evidence suggestive of infection <i>plus</i> signs of a systemic response to the infection (all of the following): Tachypnea (respiration > 20 breaths/min (if patient is mechanically ventilated, > 10 L/min)) Tachycardia (heart rate > 90 beats/min) Hyperthermia or hypothermia (core or rectal temperature > 38.4 °C [101 °F] or < 35.6 °C [96.1 °F])
The sepsis syndrome (may also be considered <i>incipient septic shock</i> in patients who later become hypotensive)	Clinical diagnosis of sepsis outlined above, <i>plus</i> evidence of altered organ perfusion (one or more of the following): Pao ₂ /Fio ₂ no higher than 280 (in the absence of other pulmonary or cardiovascular disease) Lactate level above the upper limit of normal Oliguria (documented urine output < 0.5 mL/kg body weight for at least 1 hour in patients with catheters in place) Acute alteration in mental status Positive blood cultures are not required‡
Early septic shock	Clinical diagnosis of sepsis syndrome outlined above, <i>plus</i> hypotension (systolic blood pressure below 90 mm Hg or a 40 mm Hg decrease below baseline systolic blood pressure) that lasts for less than 1 hour and is responsive to conventional therapy (intravenous fluid administration or pharmacologic intervention)
Refractory septic shock	Clinical diagnosis of the sepsis syndrome outlined above, <i>plus</i> hypotension (systolic blood pressure below 90 mm Hg or a 40 mm Hg decrease below baseline systolic blood pressure) that lasts for more than 1 hour despite adequate volume resuscitation and that requires vasopressors or higher doses of dopamine (> 6 µg/kg per hour)

* Adapted from Reference 5.

† The related term *septicemia* is imprecise and should be abandoned.

‡ The sepsis syndrome may result from infection with gram-positive or gram-negative bacteria, pathogenic viruses, fungi, or rickettsia; however, an identical physiologic response may result from such noninfectious processes as severe trauma or pancreatitis. Blood cultures may or may not be positive.

that the incidence of septicemia (which they define as systemic disease associated with the presence and persistence of pathogenic microorganisms or their toxins in the blood) increased 139% between 1979 and 1987, from 73.6 to 175.9 cases per 100 000 persons (3). Septicemia is now ranked as the thirteenth leading cause of death in the United States (3, 6) and is estimated to account for \$5 to \$10 billion of American's annual health care expenditures (3).

The frequency with which shock develops in patients with sepsis varies. Martin and coworkers (2) found a 17% incidence of shock in patients with coagulase-negative staphylococcal infection. In contrast, shock developed in 47% of the bacteremic patients and 30% of the nonbacteremic patients in the placebo group of the Methylprednisolone Severe Sepsis Study (7). Overall estimates suggest that shock develops in about 40% of patients with sepsis (8).

The onset of sepsis—particularly septic shock—portends a poor prognosis. In a study of 487 patients consecutively admitted to a medical intensive care unit, sepsis developed in 196 (40%) (9). Forty-four percent (86 of 196) of the septic patients died, but only 16% (45 of 291) of the patients with ut sepsis died. Other studies have estimated that the mortality associated with sepsis ranges from about 10% to 20% (10) up to 50% (11, 12). (As noted above, variations in definition may account for at least some of the variations in mortality rates.) The onset of shock may increase mortality to as high as

77% (13) or 90% (1). In our studies, the sepsis syndrome without shock had a mortality of 13%, the sepsis syndrome presenting with shock had a mortality of 28%, and shock developing after the sepsis syndrome had a mortality of 43% (7).

Pathophysiology

The definitions presented in Table 1 are useful clinically and experimentally, because they allow us to identify patients and to assess the outcome of treatment in a uniform fashion. Nevertheless, sepsis, the sepsis syndrome, and septic shock are not discrete entities; rather, these terms delineate increasingly severe stages of the same disease and result from the same pathophysiologic processes. For simplicity's sake, I will use the term "sepsis" in much of the remainder of this article to denote all three disorders. I will ultimately explain, however, how these pathophysiologic processes contribute to the different stages of disease severity.

The mediators of sepsis that are most likely to produce endothelial dysfunction are listed in the Appendix. Also included in the Appendix are the effects of these mediators that are most likely to contribute to the development of sepsis.

As detailed as this Appendix may seem, several limitations should be noted. First, many of the mediators of sepsis probably remain undiscovered. Second, our knowledge of the mediators that have been discovered

is far from complete, but we do know that many of these mediators exert effects that may be protective of the host in one setting but injurious in another (14, 15). Third, these mediators interact in a very complex manner; although interactions are often synergistic, at times they may be quite antagonistic. Although still incompletely understood, this nexus can be seen as a cascade that is initiated by a focus of infection or injury and ends with severe endothelial damage, profound hemodynamic derangements and, often, death.

The initiating event in this "sepsis cascade" is the release of endotoxin or a comparable substance into the circulation (Figure 1). (I use endotoxin in this discussion because gram-negative bacteria are frequently the cause of sepsis and because much of the work that has been done to elucidate these mediators was done using endotoxin.) Enterotoxin, toxic shock syndrome toxin-1, gram-positive or yeast cell-wall products, and viral or fungal antigens, however, can also initiate the sepsis cascade (16, 17). Noninfectious stimuli can also produce an identical physiologic response. Whether this response can truly be called "sepsis," however, has been debated.

Once in the circulation, endotoxin prompts the release of tumor necrosis factor α (TNF α), interleukin 1, interleukin-6, interleukin-8, and platelet-activating factor (PAF) from mononuclear phagocytes and other cells, including the endothelial cells themselves (18-21). The coagulation cascade and complement system become activated, although it is not clear whether this is a direct result of endotoxin (12, 22), stimulation by TNF α (23-26), or another mediator (27-30), or both.

After release of TNF α , interleukin-1, and PAF, arachidonic acid is metabolized to form leukotrienes, thromboxane A₂, and prostaglandins (especially prostaglandin E₂ [PGE₂] and prostaglandin I₂ [PGI₂]) (31). Interleukin-1 and interleukin-6 activate the T cells to produce interferon- γ , interleukin-2, interleukin-4, and granulocyte-monocyte colony-stimulating factor (14, 32-34).

Almost all of these agents have direct effects on the vascular endothelium. Endotoxin (16), TNF α (16, 35), PAF (36), leukotrienes (37), and thromboxane A₂ (31) each increase endothelial permeability. The vascular endothelium also seems to be the primary target for interleukin-1-induced changes in sepsis (14).

The endothelium, in turn, releases two additional substances, endothelium-derived relaxing factor (EDRF) (38, 39) and endothelin-1 (39, 40). These two substances seem to counterbalance each other; EDRF relaxes smooth muscle and inhibits platelet aggregation (41), whereas endothelin-1 is a potent vasoconstrictor (40).

Activation of the complement cascade (particularly fragments C3a and C5a [42]) results in vascular abnormalities (43-45) and neutrophil activation (46, 47). Neutrophils may also be activated directly by most of the mediators listed above (28, 26, 29, 31, 48-50). As a result, neutrophil-induced damage may occur during degranulation (through release of free radical oxygen species and lysosomal enzymes), during aggregation (through microemboli formation), and during adherence to the endothelium (through vasodilation) (14, 47).

Platelets may also be involved in the sepsis cascade, although the evidence for this is less clear. They may

damage the endothelium in two ways: by inducing vasoconstriction and by neutrophil stimulation (51). A derivative of platelets, transforming growth factor β_1 , may also be involved (52).

Other agents that may be part of the sepsis cascade include adhesion molecules (53), kinins (54-56), thrombin (57-59), myocardial depressant substance (60-62), beta-endorphin (63, 64), and heat shock protein (65). Adhesion molecules and thrombin may help promote endothelial damage; interleukin-4, interleukin-8, and heat shock protein may protect against it. It is not yet clear, however, exactly how these agents are activated or what specific role they play.

A Central Mediator of Sepsis?

As complicated as this cascade may seem, it may actually oversimplify the clinical picture. Because most of these mediators can prompt release of the others (and, in at least some cases, of themselves), it is often difficult to ascribe a given effect to a specific mediator. Further, it has recently been found that many of these mediators have different effects in combination than they have individually (66). It has thus been extraordinarily difficult to find the mediator (or mediators) that is most directly responsible for sepsis, but it has been hoped that the discovery of such a central mediator would permit development of more effective treatment regimens.

Toward this goal, a great deal of attention has recently focused on the role of TNF α . The arguments put forth for why this mediator should be considered central can be summarized as follows:

1. Tumor necrosis factor α levels are often elevated in a variety of septic states (67-76).
2. Many of the invasive stimuli known to cause sepsis, such as endotoxin and enterotoxin, prompt macrophages to release TNF α (16).
3. Injection of a lethal dose of endotoxin causes a sharp rise in TNF α levels in animals (77); injection of sublethal doses causes a smaller, but still marked, increase in TNF α levels in healthy human volunteers (76, 77-79).
4. Administration of large doses of TNF α duplicates many of the signs and symptoms of septic shock, including hypotension, neutropenia, and increased edema and pulmonary permeability (35).
5. Administration of antibodies to TNF α protects against the lethal effects of subsequent endotoxin challenge (80-82).

Unfortunately, as elegant and as seemingly persuasive as this argument is, it has several flaws. First, as our knowledge of TNF α has increased, it has become clear that TNF α levels are elevated in a wide variety of conditions other than sepsis. From its earliest discovery, TNF α was known to lyse cancer cells (83), and many cancer patients have been found to have increased circulating TNF α levels (84, 85). Elevated TNF α levels are also associated with various arthritides, including rheumatoid arthritis (86) and systemic lupus erythematosus (87), and also with the acquired immunodeficiency syndrome (88), leprosy and leishmaniasis (89, 90), coal workers' pneumoconiosis (79), and

alcoholic hepatitis (91). Raised $\text{TNF}\alpha$ levels have also been found in patients with congestive heart failure (92). Thus, $\text{TNF}\alpha$, at best, can be considered a nonspecific mediator of inflammation with additional noninflammatory roles. The $\text{TNF}\alpha$ levels found in patients with these other disorders are often not markedly different from those found in most patients with sepsis.

Further, many septic patients do not have elevated—or even detectable— $\text{TNF}\alpha$ levels (93, 70-72), and many healthy persons have measurable levels of $\text{TNF}\alpha$ (72-74). One study reported that mean $\text{TNF}\alpha$ levels in 124 normal persons was 75.3 ± 15.6 pg/mL (73). Consequently, it is difficult to determine exactly what

should be considered a “normal” $\text{TNF}\alpha$ level. When we re-evaluate the studies of $\text{TNF}\alpha$ levels in septic patients in light of these data, it becomes more difficult to conclude that $\text{TNF}\alpha$ levels are increased sufficiently so that they can be considered responsible for most of the findings in sepsis.

Some investigators have argued that the phasic nature of $\text{TNF}\alpha$ release makes it difficult to detect elevated plasma levels (17). I believe that we must think of $\text{TNF}\alpha$ as a paracrine, not an endocrine, hormone (that is, it is a hormone that exerts predominantly local, rather than systemic, effects); high circulating levels of $\text{TNF}\alpha$ may never be present in many patients.

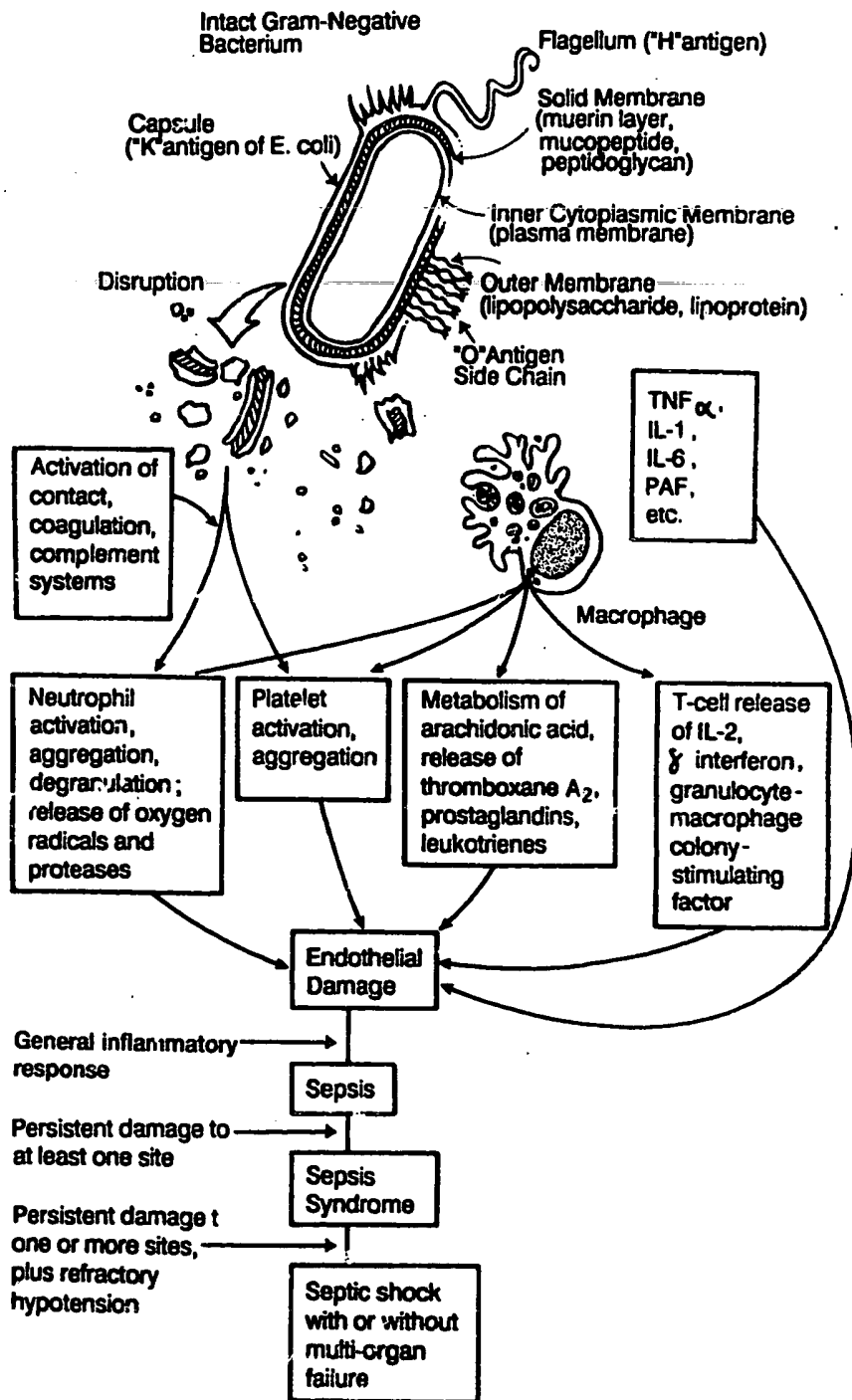


Figure 1. Schematic of sepsis. Note that although the schematic illustration somewhat oversimplifies the pathophysiologic process, it does provide a framework for understanding a complex chain of events. The pathways are not distinct, and the effects of any one mediator may vary with physiologic conditions. Massive endothelial damage does not ordinarily occur because there are many points at which feedback loops downregulate mediator release. If the body cannot restore homeostasis, however, the generalized inflammatory response will produce clinical evidence of sepsis. Persistent endothelial damage to at least one site will produce signs of organ failure (the sepsis syndrome); if hypotension develops and proves refractory to treatment, the patient will meet the criteria for septic shock.

Second, we must be careful when extrapolating the results of animal studies to the clinical setting. There are significant cross-species differences in response to endotoxin and other mediators (94). Further, most cytokines are species-specific and thus administration of a human cytokine (or antibody to that cytokine) may not produce an accurate response in an animal model (95, 96). Differences in experimental techniques and methods of TNF α measurement cloud the picture still further.

We must also be cautious when interpreting the results of experiments in human volunteers. By definition, these volunteers are healthy, whereas patients with septic shock are severely ill, immunocompromised, or both, even before shock sets in. Additionally, such studies must use an artificial dose of endotoxin—one that is large enough to cause a measurable response but low enough not to cause death. This dose is generally administered as a single intravenous bolus, which is probably not comparable to the pattern of release in sepsis.

Third, administration of antibodies to endotoxin also protects against the lethal effects of subsequent endotoxin challenge, but in animal models it does not lower the rise in TNF α levels caused by endotoxin administration (97). Also, in mice infected with *Histoplasma capsulatum*, administration of antibodies to TNF α accelerates death (98).

Finally, TNF α is not the only mediator that can replicate the symptoms of sepsis. Administration of low doses of either interleukin-1 or PAF, for example, has been shown to induce a shocklike state (99-101). Unfortunately, many of the same arguments that can be made against TNF α as the central mediator of sepsis can also be made against interleukin-1 and PAF. Elevated levels of interleukin-1 can be found in patients with inflammatory bowel disease (102), systemic juvenile chronic arthritis (103), rheumatoid arthritis (104), and pulmonary fibrosis and sarcoidosis (105). Platelet-activating factor has been detected in patients with asthma (106) and kidney disease (107).

This is not to say that TNF α (or interleukin-1 or PAF) does not have a role in the pathogenesis of sepsis. It does. It may well be one of the most important mediators in the sepsis cascade, but its effects occur predominantly at a local level. High circulating titers of TNF α may portend poor prognosis for patients with septic shock (68, 69, 71, 75, 76), although this has been questioned by some (72). Too many patients with too many different disorders, however, have elevated TNF α levels without suffering circulatory collapse, and too many patients with sepsis have no or only low TNF α levels. High circulating levels of TNF α are thus not a prerequisite for the development of sepsis.

Mediator Interactions

If there seems to be no central mediator that explains the origins of sepsis, we must consider whether there is a specific interaction between two or more mediators that provides the prime impetus. The mediators in the sepsis cascade interact in various ways. Some interactions seem to augment the host response to infection. Interferon- γ and TNF α , for example, can enhance the

phagocytic activity of neutrophils (26). Other interactions appear to limit inflammation, restore homeostasis, or both. The cycle in which TNF α prompts PGI $_2$ release (31) and then PGI $_2$ suppresses further TNF α synthesis (108) is one example. Another is the process by which interleukin-8 decreases neutrophil adherence to the endothelium (109). Similarly, the products of the neutrophilic burst can inactivate leukotrienes at inflammatory sites (110). The vasodilatory effects of EDRF counterbalance the vasoconstrictive influence of endothelin-1 (39, 111).

The interactions of other mediators, however, seem more likely to disrupt homeostasis. For example, TNF α , interleukin-1, and PAF are able to promote release of one another—and their own continued release (112). Activation of T cells (mediated in large part by interleukin-1) causes release of interferon- γ ; interferon- γ , in turn, stimulates macrophages to release more interleukin-1 (27). Both TNF α and PAF promote arachidonate metabolism, but arachidonate derivatives can then prompt TNF α and PAF release (31).

Further, each of the mediators of sepsis can be produced by a wide variety of cells in many different parts of the body. Both TNF α and interleukin-1, for example, can be released by macrophages, lymphocytes, natural killer cells, astrocytes and microglial cells, Kupffer cells, and endothelial cells (20, 21, 32, 99, 113, 114). Platelet-activating factor can be produced by macrophages, neutrophils, eosinophils, endothelial cells, and platelets (29, 115-117). As a consequence, the number of interactions between mediators may be close to the number of mediators squared.

It is precisely because of the complexity of this cascade that it has been difficult to elucidate exactly what effects can be ascribed to each mediator. Moreover, many mediators have synergistic effects. Interleukin-1 and TNF α act synergistically in disrupting pulmonary vascular endothelium (99). Endotoxin has been shown to potentiate the effects of TNF α (118).

Most important, it has been found that the actions of a given mediator may differ from setting to setting. Three factors are responsible for this variability (66):

1. *The state of activation of the target cell.* Interferon- γ , for example, prompts proliferation of quiescent fibroblasts but inhibits proliferation when fibroblasts are rapidly dividing (66). Prostaglandin E $_2$ stimulates release of TNF α from resting monocytes (110) but it inhibits TNF α production once the macrophages are activated (119).

2. *The nearby presence of other mediators.* Administration of TNF α cannot mimic the effects of endotoxic shock unless PAF is present (120, 121). Neutrophil generation of superoxide anions can be attenuated in the presence of platelets but not in platelet-free environments (122).

3. *The ability of the target cell to release other mediators.* Tumor necrosis factor α and interleukin-1 each cause fibroblast proliferation, but the combination of the two synergistically produces PGE $_2$ release, which, in turn, blocks fibroblast proliferation (66). Endothelial cells can lose their ability to secrete EDRF following exposure to endotoxin (41); secretion is also altered by shear stress and changes in wall tension (123). Surface

expression of adhesion molecules is both site-specific and time-dependent (53, 124).

Other Variables

Three other variables must be considered in any discussion of the role of these mediators in sepsis: the clinical status of the patient before the onset of sepsis, the length of time the patient has been ill, and innate variations in patients' ability to secrete these mediators.

Severe sepsis and septic shock do not develop in healthy persons; in most instances, they develop in persons with preexisting severe disease or in persons who have suffered catastrophic acute illness or trauma. This point may seem obvious but should not be overlooked. Patients at greatest risk of dying of sepsis are the elderly; those receiving immunosuppressive drugs; and those with malignancies, cirrhosis, asplenia, or multiple underlying disorders (such as diabetes mellitus, renal failure, or heart disease). As described above, however, these patients often already have elevated levels of one or more inflammatory mediators. They may also have activated macrophages, neutrophils, and T cells, and thus are "primed" for the release of additional mediators.

Production of other mediators, however, may sometimes be reduced in such patients. For example, vascular tissue is less able to generate PGI_2 in elderly patients, in patients with diabetes, and in patients with atherosclerosis (39); this decrease in PGI_2 generation may contribute to the loss of EDRF in these patients. Increased production of some mediators coupled with decreased production of others may make it difficult for the body to restore homeostasis.

The length of the patient's illness may also alter the "mix" of mediators. Synthesis of cytokines may be decreased after continued infection, downregulation of the relevant receptor may occur, or inhibitors may eventually be generated. This may help explain why high circulating levels of some mediators in a susceptible patient do not produce the massive toxic response that occurs in a healthy volunteer.

There may also be innate differences in patients' response to (or ability to produce) $\text{TNF}\alpha$ and other mediators. After injection of endotoxin, human subjects show marked variation in $\text{TNF}\alpha$ levels and response to fever (78, 125). The finding of increased cytokine levels in patients with various arthritides or inflammatory bowel disease further supports the concept that some patients may be inherently more susceptible to cytokine-induced damage.

Endothelial Dysfunction, Sepsis, and Septic Shock

How then do these mediators contribute to the development of sepsis? As noted above, sepsis usually begins with a nidus of infection or injury. At the site of original involvement, endotoxin, enterotoxin, or another stimulus prompts macrophages to release cytokines, PAF, eicosanoids, and T- and B-cell products. These products, however, are most likely to be found in the immediate microenvironment; they may be absent in other areas.

In the microenvironment, the beneficial effects of

these mediators probably outweigh the negative effects; they enhance host defenses against infection and contribute to tissue remodeling (21). If the infection is not brought under control, however, one or more of these mediators—or endotoxin itself—may leak into the circulation.

Once in the circulation, endotoxin or $\text{TNF}\alpha$ (but probably many other mediators as well) is capable of triggering the full sepsis cascade. Whether this occurs depends on several factors, including the nearby presence or absence of other mediators released into or already present within the circulation, the nearby presence or absence of macrophages and activated neutrophils in the circulation, the relative health of the patient's vital organs (other than the infected organ or organs), and the underlying status of the patient's endothelium.

In the best clinical scenario, the release of endotoxin or mediator into the circulation will not cause sepsis. Built into the sepsis cascade are many places at which the system can downregulate itself. Oxidative products may provide negative feedback to control inflammation (110, 126, 127). Prostaglandin E_2 release may dampen the ability of macrophages to release cytokines and other mediators (128), and macrophages may be able to actively suppress T cells (129), thereby restoring homeostasis.

In some patients, unfortunately, downregulation does not occur; too much endotoxin or mediator may have been released, appropriate mediators to promote downregulation may not be present, or too many other mediators are released before downregulation begins. At this point, the patient will have begun to demonstrate clinical evidence of a systemic response to infection, thereby meeting the definition for sepsis presented in Table 1.

Inflammatory mediators will continue to circulate until they become inactive or until they reach the capillaries. In the capillaries, they may begin to cause endothelial damage through the mechanisms described above. Should sufficient damage be done to any one site, the patient will show signs of organ failure, thereby fulfilling the criteria for the sepsis syndrome.

The endothelium has an elaborate defense system. Like macrophages, endothelial cells are able to secrete $\text{TNF}\alpha$, interleukin-1, interleukin-6, and PAF (99, 114, 115, 130), as well as the metabolites of arachidonic acid (38, 131-133) and also EDRF and endothelin-1 (39). Given the short half-lives of many of these mediators (thromboxane A_2 and PGI_2 have half-lives of about 30 seconds; EDRF, approximately 6 seconds), endothelial release of these substances may well be more important than macrophageal release in the pathogenesis of sepsis. Again, the initial effects of the mediators may be beneficial: Endothelial damage is repaired and the patient recovers.

If the endothelium cannot repair itself and additional mediators are released into the circulation, however, more sites of damage will develop. Ultimately, the blood pressure drops. The reason that hypotension occurs is not fully understood; it may result from the effects of many of these mediators (especially $\text{TNF}\alpha$) on the heart, from the effects of a more specific mediator

(such as myocardial depressant substance), from the augmented release of EDRF or bradykinin, or from some combination of the above. Once hypotension develops, the patient fulfills the criteria for septic shock.

The sequence of events I have outlined can best be envisioned as a pyramid (Figure 2), in which each level reflects progressively greater degrees of endothelial damage. It is important to remember, however, that the same factors that contribute to endothelial damage in one organ may simultaneously or sequentially promote de-endothelialization elsewhere. Further, each site of inflammation may release yet more mediators into the circulation, thereby increasing the likelihood that additional damage will be done.

At each of these sites, a different mix of mediators may be responsible for endothelial destruction. In addition, the extent of damage may differ at each site. In some places, inflammation may be inhibited and homeostasis restored; in other places, endothelial destruction may continue unabated until organ failure ensues.

Implications

This model has important implications for diagnosis and therapy. First, the development of sepsis or its sequelae does not require the persistent release of endotoxin into the circulation; many of the mediators may initiate the process. This may help explain why many septic patients are never shown to be bacteremic. Second, as discussed, the amount of endotoxin or mediator released initially does not need to be large; small amounts of TNF α or PAF, for example, may be more than capable of causing sepsis in the setting of neutrophil activation and endothelial damage. Further, the continued presence of the instigating mediator is not required for sepsis to develop.

Once organ damage occurs, however, local release of mediators can be quite extensive. Bronchoalveolar lavage fluid specimens from patients with the adult respi-

ratory distress syndrome, for example, have been shown to have TNF α concentrations greater than 500 U/mL (12 500 pg/mL) (134), a level dramatically higher than the highest serum levels detected in patients with septic shock.

Third, different mediators may be the initial stimulus in different patients. This theory may help explain why different organs are affected initially in different patients. For example, TNF α binds preferentially to the kidney, lung, and liver (135, 136); PAF often contributes to gastrointestinal ulceration (137); and the kidneys are more sensitive to the effects of endothelin-1 than are the other organs (138).

Fourth, if sufficient endothelial damage occurs, new organisms may be allowed into the bloodstream. This may occur most often in the gastrointestinal tract; bacterial translocation may promote the release of yet additional mediators. Extensive damage to any one organ will also hinder the body's ability to respond to ongoing infection. For example, damage to the pulmonary vasculature will alter the body's ability to remove endothelin-1 from the circulation (111). Alveolar macrophages have also been shown to gradually lose their ability to kill cell-associated *Staphylococcus aureus* and *Escherichia coli* (139). Hepatic damage may disrupt the reticuloendothelial system and limit the systemic response to sepsis (140).

Finally, any treatment plan for sepsis must take into account the fact that at any given time, a patient will have multiple, often persistent sites of inflammation, each at a different stage. Thus, by the time a patient has the sepsis syndrome or septic shock, monotherapy is unlikely to be effective. Most patients will require a host of treatments, and the earlier therapy is started, the more likely it will be effective.

What if treatment is not given in time? I believe that the sepsis cascade can eventually become self-perpetuating, independent of the original mediator or media-

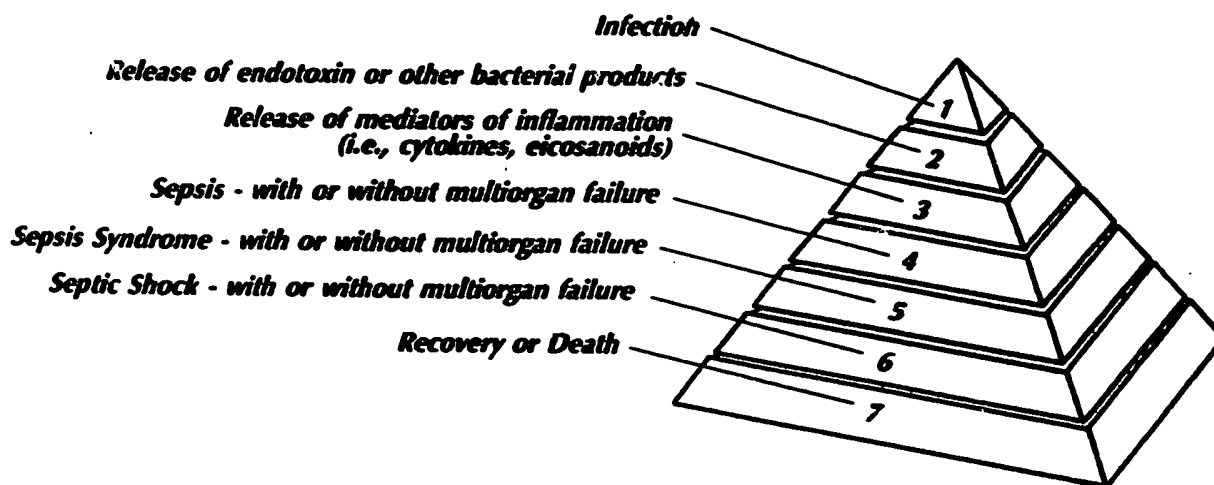


Figure 2. Model of sepsis. The mediators of sepsis can be shown to produce an expanding sequence of events according to the intensity or dose of the original insult. This is the model found in many experimental models of sepsis. The clinical syndrome of sepsis usually evolves over a longer time interval and causes a continuing insult. The systemic result is altered not only by the intensity of the response but by previous insults and earlier compensatory efforts (for example, the production of cytokine inhibitors or downregulators of relevant receptors). As a consequence, the inflammatory process is repeated at different sites; each site may be at a different stage of inflammation.

tors. Multiorgan failure ensues as more and more sites of endothelial damage escape control. The reticuloendothelial system eventually becomes hyperstimulated; macrophages lose the ability to secrete TNF α (18) and,

possibly, interleukin-1 (141). The net result is a state of metabolic anarchy in which the body can no longer control what it has created. Unless effective treatment is given, too often the patient will die.

Appendix. Mediators of Endothelial Damage in Sepsis

Mediator	Major Reported Effects
Tumor necrosis factor α (TNF α)	<p>Stimulates release of interleukin-1, interleukin-6, interleukin-8, platelet-activating factor, leukotrienes, thromboxane A₂, prostaglandins; may be able to stimulate macrophages directly to promote its own release</p> <p>Has only a weak effect on T cells</p> <p>Stimulates production of polymorphonuclear cells by bone marrow; enhances phagocytic activity of polymorphonuclear cells</p> <p>Promotes adhesion of endothelial cells, polymorphonuclear cells, eosinophils, basophils, monocytes, and, occasionally, lymphocytes by inducing increased expression of adhesion molecules</p> <p>Activates common pathway of coagulation and complement system</p> <p>Is directly toxic to vascular endothelial cells; increases microvascular permeability</p> <p>Produces a dose-dependent increase in endothelial procoagulant activity; may inhibit thrombomodulin expression at endothelial cell surface</p> <p>Suppresses lipoprotein lipase activity; inhibits acetate by adipocytes; decreases incorporation of glucose</p> <p>Stimulates collagenase release; has variable effects on fibroblast growth, depending on the presence of other mediators</p> <p>Reduces transmembrane potential of muscle cells and depresses cardiac myocyte shortening</p> <p>Induces class I histocompatibility molecules</p> <p>Acts directly on hypothalamus to produce fever</p>
Interleukins	
Interleukin-1	<p>Stimulates release of TNFα, interleukin-6, interleukin-8, platelet-activating factor, leukotrienes, thromboxane A₂, prostaglandins; may also be capable of stimulating its own production</p> <p>Activates resting T cells to produce lymphocytes and other products; supports B-cell proliferation and antibody production; is cytotoxic for insulin-producing B cells</p> <p>Promotes adhesion of endothelial cells, polymorphonuclear cells, eosinophils, basophils, monocytes, and, occasionally, lymphocytes by inducing increased expression of adhesion molecules</p> <p>Promotes polymorphonuclear cell activation and accumulation</p> <p>Increases endothelial procoagulant activity and endothelial release of plasminogen activator inhibitor</p> <p>Acts synergistically with TNFα; enhances tissue cell sensitivity to TNFα</p> <p>Suppresses lipoprotein lipase activity</p> <p>Encourages collagenase release from synovial cells; affects fibroblast proliferation and stimulation of its secretory products</p> <p>Promotes release of adrenocorticotrophic hormone</p> <p>Acts directly on hypothalamus to produce fever</p>
Interleukin-2	<p>May promote release of TNFα and interferon-γ</p> <p>Required for proliferation of activated T cells</p> <p>Decreases arterial pressure, systemic vascular resistance, and ejection fraction; increases cardiac output</p>
Interleukin-4	<p>Enhances lymphocyte adhesion to endothelial cells</p> <p>Regulates growth or differentiation of hematopoietic factors, including T cells and mast cells</p> <p>Induces antigen expression on macrophages</p> <p>Suppresses interleukin-8 expression from stimulated monocytes but not from stimulated fibroblasts or endothelial cells</p> <p>Synergistically increases TNFα- or interleukin-1-induced antigen expression on endothelial cells, but inhibits the increased expression of adhesion molecules by TNFα, interleukin-1, or interferon-γ</p>
Interleukin-6	<p>Acts as a helper cell for T- and B-cell activation; interacts synergistically with interleukin-1 to affect thymocyte proliferation; in combination with TNFα, augments T-cell proliferation</p> <p>Promotes polymorphonuclear cell activation and accumulation</p>
Interleukin-8	<p>Is chemotactic for both neutrophils and lymphocytes; induces tissue infiltration of both</p> <p>Inhibits endothelial-leukocyte adhesion; decreases the hyperadhesion induced by those molecules</p>
Platelet-activating factor	<p>Stimulates release of TNFα, leukotrienes, thromboxane A₂</p> <p>Promotes leukocyte activation and subsequent free-radical formation</p> <p>Encourages platelet aggregation leading to thrombosis</p> <p>Markedly alters microvascular permeability, thereby promoting microvascular fluid loss</p> <p>Stimulates calcium influx-efflux in endothelial cells; causes such cells to retract and lose reciprocal contact (allowing exposure of the subintimal tissue to the blood stream), and promotes albumin diffusion in endothelial cells</p> <p>Exerts a negative inotropic effect on the heart; lowers arterial blood pressure</p> <p>May attenuate effects of endothelin on hyperglycemia and hyperlactacidemia</p> <p>May cause gastrointestinal ulceration, particularly in the duodenum and jejunum</p> <p>Induces blood-brain damage and vasoconstriction; may be neurotoxic</p>

Appendix. Mediators of Endothelial Damage in Sepsis (Continued)

Mediator	Major Reported Effects
Leukotrienes Leukotriene B ₄	Promotes neutrophil chemotaxis and adhesion of neutrophils to endothelium (neutrophils have specific receptors for leukotriene B ₄) Is weakly chemotactic for eosinophils Increases vascular permeability, either directly or through interaction of neutrophils and endothelial cells
Leukotriene C ₄ , leukotriene D ₄ , and leukotriene E ₄	Stimulate release of prostacyclin Increase vascular permeability directly by causing contraction of adjacent endothelial cells and a resulting increase in the diameter of interendothelial-cell pores Decrease coronary blood flow and myocardial contractility; increase pulmonary vascular resistance; decrease mesenteric blood flow but have no effect on renal blood flow Have mild vasoconstrictive effects themselves and enhance vasoconstrictive effects of epinephrine and norepinephrine; however, can relax precontracted arterial segments (only when endothelial lining is intact)
Thromboxane A ₂	Promotes release of endothelium-derived relaxing factor; may stimulate prostacyclin production Causes platelet aggregation and neutrophil accumulation Increases vascular permeability; enhances permeability of both single- and double-unit membranes Produces vasoconstriction of vascular beds and pulmonary bronchoconstriction
Prostaglandins Prostaglandin E ₂	Inhibits both interleukin-1 production and the responsiveness of thymocytes to interleukin-1 Low concentrations stimulate TNF α release; higher concentrations suppress TNF α production at a dose-dependent level Inhibits mitogenesis of T and B cells Causes vasodilation and increased blood flow Has a beneficial effect on tissue perfusion and may thereby decrease the severity of tissue damage Acts synergistically with prostacyclin to increase the effects of serotonin and bradykinin on vascular permeability Promotes muscle catabolism Inhibits (at a dose-dependent level) endotoxin's ability to produce hypotension and gastric damage Increases intracellular cyclic AMP levels Acts directly on hypothalamus to produce fever
Prostacyclin (Prostaglandin I ₂)	Inhibits platelet aggregation and adhesion (this effect may be synergistically increased by endothelium-derived relaxing factor) Inhibits thrombus formation; may have fibrinolytic activity Acts synergistically with prostaglandin E ₂ to increase the effects of serotonin and bradykinin on vascular permeability Causes vasodilation and increased blood flow In the early stages of sepsis, exerts a beneficial effect on tissue perfusion Produces smooth muscle relaxation
Interferon- γ	Promotes release of TNF α , interleukin-1, interleukin-6 (possibly due to its ability to augment effects of endotoxin on macrophages); augments production of adhesion molecules May act synergistically with TNF α to produce cytotoxic and cytostatic activity; interacts with other cytokines in variable ways Synergistically increases interleukin-2 promotion of TNF α release Helps activate B cells to increase antibody production Enhances adhesion of lymphocytes to endothelial cells Produces marked morphologic changes in endothelial cells Encourages polymorphonuclear cell activation and accumulation; enhances the phagocytic activity of polymorphonuclear cells Promotes macrophage activation, macrophage microbicidal function, and expression of cellular receptors for TNF α Induces class I and class II histocompatibility molecules May antagonize production of granulocyte-monocyte colony-stimulating factor Acts directly on hypothalamus to produce fever
Granulocyte-monocyte colony-stimulating factor	Stimulates polymorphonuclear cell phagocytosis, degranulation, and cytotoxicity Promotes macrophage maturity and enhances macrophage activity
Endothelium-derived relaxing factor	Relaxes vascular smooth muscle Inhibits platelet aggregation and adhesion (this effect may be synergistically increased by prostacyclin) Inhibits mitogenesis in vascular smooth muscle cells
Endothelin 1	Strongly promotes vasoconstriction By increasing glomerular resistance, may cause renal hypoperfusion and hypofiltration Promotes release of endothelium-derived relaxing factor and prostacyclin Encourages mitogenesis in vascular smooth muscle cells

(Continued on next page)

Appendix. Mediators of Endothelial Damage in Sepsis (Continued)

Mediator	Major Reported Effects
Complement fragment C3	Causes mast cells to degranulate and release vasodilatory mediators May promote endotoxin-stimulated release of thromboxane A ₂ and prostacyclin Causes smooth muscle contraction and mucus secretion
Complement fragment C5 _a	Causes mast cells to degranulate and release vasodilatory mediators May promote endotoxin-stimulated release of thromboxane A ₂ and prostacyclin Promotes TNF α release Enhances polymorphonuclear cell activation, migration, adherence, and aggregation Induces capillary leakage May depress systemic vascular resistance and produce hypotension
Polymorphonuclear cells	Can release directly, or indirectly promote release of, most of the mediators described above Have variable effects on platelet aggregation During degranulation, release free-oxygen species and lysosomal enzymes, which, in turn, may: A. Damage vascular endothelium, mitochondria, and collagen B. Cause polymorphonuclear cell dysfunction, erythrocyte fragility, intravascular hemolysis, and vascular smooth muscle contraction C. Enhance smooth muscle sensitivity to alpha-adrenergic agonists D. Counteract the effects of endothelium-derived relaxing factor During aggregation: A. Create microemboli, which contribute to arteriole occlusion and gross peripheral maldistribution of blood flow During adherence to endothelial wall: A. Produce a variable effect on tone in vessels with intact endothelium, depending on concentration B. Produce dose-dependent smooth muscle relaxation in de-endothelialized vessels C. Release endothelium-derived relaxing factor-like substance called neutrophil-derived relaxing factor D. Migrate through interendothelial cell junctions and accumulate in subcellular regions
Adhesion molecules	Act as ligands for receptors on inflammatory cells
Endothelial-leukocyte adhesion molecule-1	Attracts neutrophils to vascular endothelium
Intercellular adhesion molecule-1	Attracts neutrophils and B lymphocytes (but not T lymphocytes) to vascular endothelium Helps neutrophils and T lymphocytes to transmigrate across endothelial monolayers
Vascular cell adhesion molecule-1	Mediates binding of monocytes and lymphocytes to vascular endothelium
Platelets	Promote release of endothelium-derived relaxing factor, prostacyclin Produce transforming growth factor- β_1 , serotonin, thromboxane A ₂ ; also release 12-hydroxy acids that are metabolized by neutrophils into leukotriene B ₄ Induce vasoconstriction and stimulate neutrophils (both effects require release of other mediators)
Transforming growth factor β_1	Recruits macrophages to area of injury, then stimulates them to release interleukin-1 and more transforming growth factor- β_1 May suppress macrophages' ability to release reactive oxygen species and to promote leukocyte adherence to endothelium May suppress TNF α -induced inflammation Promotes cell growth, repair, metabolism; increases production of extracellular matrix by mesenchymal cells Causes intestinal smooth muscle cells to double collagen production but has no effect on cell proliferation
Bradykinin	Promotes release of endothelium-derived relaxing factor, prostacyclin
Thrombin	Stimulates release of platelet-activating factor from human endothelial cells; disrupts platelet-activating factor regulation Promotes release of endothelium-derived relaxing factor (this may decrease with age) and prostacyclin; also encourages endothelin-1 release Mediates the activation of interleukin-8 in the procoagulant environment associated with endothelial cells Encourages fibrinogen consumption and pulmonary vasoconstriction Protein C activator (a derivative of thrombin) inactivates coagulation factors V _a and VIII _a

(Continued on next page)

Appendix. Mediators of Endothelial Damage in Sepsis (Continued)

Mediator	Major Reported Effects
Myocardial depressant substance	Produces reversible myocardial depression, ventricular dilation, decreased left ventricular ejection fraction May cause ventricular diastolic pressure abnormalities Does not seem to affect afterload or heart rate
β -Endorphin	May contribute to glucose-mediated hyperinsulinism May cause hypotension
Heat shock proteins	Prevent and repair cellular injury after hyperthermia and oxidative stress Induce tolerance to subsequent stress Cannot be induced during endotoxin challenge

Requests for Reprints: Roger C. Bone, MD, Rush Medical College, 1753 W. Congress Parkway, Chicago, IL 60612.

Current Author Address: Dr. Bone: Rush Medical College, 1753 W. Congress Parkway, Chicago, IL 60612.

References

- Parker MM, Parrillo JE. Septic shock: Hemodynamics and pathogenesis. *JAMA*. 1983;250:3324-7.
- Martin MA, Plücker MA, Weiss RP. Coagulase-negative staphylococcal bacteremia. *Ann Intern Med*. 1989;110:9-16.
- Increase in national Hospital Discharge Survey rates for septicemia—United States, 1979-1987. *MMWR*. 39:31-4.
- Cunha RE, Parrillo JE. Myocardial dysfunction in sepsis. Recent insights. *Chest*. 1989;95:941-5.
- Bone RC. Sepsis, the sepsis syndrome, multi-organ failure: a plea for comparable definitions [Editorial]. *Ann Intern Med*. 1991;114:332-3.
- Annual Summary of Births, Marriages, Divorces, and Deaths: United States, 1988. Hyattsville, Maryland: U.S. Department of Health and Human Services, Public Health Service, CDC, 1989:7. Monthly vital statistics report. 1989;37(13).
- Bone RC, Fisher CJ Jr, Clemmer TP, Slotman GJ, Metz CA, Balk RA. Sepsis syndrome: a valid clinical entity. *Crit Care Med*. 1989;17:389-93.
- Kreger BE, Craven DE, McCabe WR. Gram-negative bacteremia. IV. Re-evaluation of clinical features and treatment in 612 patients. *Am J Med*. 1980;68:344-55.
- Tran DD, Groeneweld AB, van der Meulen J, Nauta JJ, Strack van Schijndel RJ, Thijs LG. Age, chronic disease, sepsis, organ system failure, and mortality in a medical intensive care unit. *Crit Care Med*. 1990;18:474-9.
- Dunn DL. Immunotherapeutic advances in the treatment of gram-negative bacterial sepsis. *World J Surg*. 1987;11:233-40.
- The Veteran's Administration Systemic Sepsis Comparative Study Group. Effect of high-dose glucocorticoid therapy on mortality in patients with clinical signs of systemic sepsis. *N Engl J Med*. 1987;317:659-65.
- Young LS. Gram-negative sepsis. In: Mandell GL, Douglas RG, Bennett JE, eds. *Principles and Practice of Infectious Diseases*. 3d ed. New York: Churchill Livingstone; 1990:611-36.
- Sprung CL, Caralis PV, Marcial EH, Pierce M, Gelbard MA, Long WM, et al. The effects of high-dose corticosteroids in patients with septic shock. A prospective, controlled study. *N Engl J Med*. 1984;311:1137-43.
- Jacobs RF, Tabor DR. Immune cellular interactions during sepsis and septic injury. *Crit Care Clin*. 1989;5:9-26.
- Malech HL, Galin JL. Neutrophils in human diseases. *N Engl J Med*. 1987;317:687-94.
- Tracey KJ, Lowry SF, Cerami A. Cachectin/TNF- α in septic shock and septic adult respiratory distress syndrome [Editorial]. *Am Rev Respir Dis*. 1988;138:1377-9.
- Fong Y, Lowry SF, Cerami A. Cachectin/TNF: a macrophage protein that induces cachexia and shock. *J Parenter Enterol Nutr*. 1988;12:72S-77S.
- Michalek SM, Moore RN, McGhee JR, Rosenstrech DL, Mergenhagen SE. The primary role of lymphoreticular cells in the mediation of host responses to bacterial endotoxin. *J Infect Dis*. 1980;141:55-63.
- Wolff SM. Biological effects of bacterial endotoxin in man. *J Infect Dis*. 1973;128(Suppl.):259-64.
- Johnston RB Jr. Monocytes and macrophages. *N Engl J Med*. 1988;318:747-52.
- Tracey KJ, Vlassara H, Cerami A. Cachectin/tumor necrosis factor. *Lancet*. 1989;1:1122-6.
- Harris RL, Musher DM, Bloom K, Gathe J, Rice L, Sugarman B, et al. Manifestations of sepsis. *Arch Intern Med*. 1987;147:1895-906.
- Nawroth PP, Stern DM. Modulation of endothelial cell hemostatic properties by tumor necrosis factor. *J Exp Med*. 1986;163:740-5.
- Berthiaume MP, Fober JS, Majean GR, Fiers W, Cotran RS, Glumbrone MA Jr. Recombinant tumor necrosis factor induces procoagulant activity in cultured human vascular endothelium: characterization and comparison with the actions of interleukin 1. *Proc Natl Acad Sci USA*. 1986;83:4533-7.
- Gamble JR, Harlan JM, Klebanoff SJ, Vadva MA. Stimulation of the adherence of neutrophils to umbilical vein endothelium by human recombinant tumor necrosis factor. *Proc Natl Acad Sci USA*. 1985;82:8667-71.
- Shalaby MR, Aggarwal BB, Rinderknecht E, Svedersky LP, Flaherty BS, Palladino MA Jr. Activation of human polymorphonuclear neutrophil functions by interferon-gamma and tumor necrosis factors. *J Immunol*. 1985;135:2069-73.
- Dinarello CA, Cannon JG, Wolff SM. New concepts on the pathogenesis of fever. *Rev Infect Dis*. 1988;10:168-89.
- Bentler B, Cerami A. Cachectin: more than a tumor necrosis factor. *N Engl J Med*. 1987;316:379-85.
- Benveniste J, Chignard M. A role for PAF-acether (platelet-activating factor) in platelet-dependent vascular diseases? *Circulation*. 1985;72:713-7.
- Braquet P, Touqui L, Shen TY, Vargaftig BB. Perspectives in platelet-activating factor research. *Pharmacol Rev*. 1987;39:97-145.
- Petrak RA, Balk RA, Bone RC. Prostaglandins, cyclo-oxygenase inhibitors, and thromboxane synthetase inhibitors in the pathogenesis of multiple systems organ failure. *Crit Care Clin*. 1989;5:303-14.
- Dinarello CA, Mier JW. Lymphokines. *N Engl J Med*. 1987;317:940-5.
- Thornhill MH, Kyan-Aung U, Haskard DO. IL-4 increases human endothelial cell adhesiveness for T cells but not for neutrophils. *J Immunol*. 1990;144:3060-5.
- Kuhweide R, Van Damme J, Coppens JL. Tumor necrosis factor- α and interleukin 6 synergistically induces T cell growth. *Eur J Immunol*. 1990;20:1019-25.
- Stephens KE, Ishizuka A, Larrick JW, Ratka TA. Tumor necrosis factor causes increased pulmonary permeability and edema. Comparison to septic acute lung injury. *Am Rev Respir Dis*. 1988;137:1364-70.
- Ibbotson GC, Wallace JL. Beneficial effects of prostaglandin E2 in endotoxin shock are unrelated to effects of PAF-acether synthesis. *Prostaglandins*. 1989;37:237-50.
- Lewis RA, Austen KF, Seherman RJ. Leukotrienes and other products of the 5-lipoxygenase pathway. Biochemistry and relation to pathobiology in human diseases. *N Engl J Med*. 1990;323:645-55.
- Dinerman JL, Mehta JL. Endothelial, platelet and leukocyte interactions in ischemic heart disease: insights into potential mechanisms and their clinical relevance. *J Am Coll Cardiol*. 1990;16:207-22.
- Vane JR, Anggard EE, Botting RM. Regulatory functions of the vascular endothelium. *N Engl J Med*. 1990;323:27-36.
- Miyachi T, Tomobe Y, Shiba R, Ishikawa T, Yamagawa M, Kikawa S, et al. Involvement of endothelin in the regulation of human vascular tone. *Circulation*. 1990;81:1874-80.
- Griffith TM, Lewis MJ, Newby AC, Henderson AH. Endothelium-derived relaxing factor. *J Am Coll Cardiol*. 1988;12:797-806.
- Berger M, Wetzler EM, Wallis RS. Tumor necrosis factor is the major monocyte product that increases complement receptor expression on mature human neutrophils. *Blood*. 1988;71:151-8.
- Ogishima FP, Parker MM, Burch-Whitman C, et al. Neutrophil aggregating activity and septic shock in humans: neutrophil aggregation by a C5a-like material occurs more frequently than comple-

- ment component depletion and correlates with depression of systemic vascular resistance. *J Crit Care.* 1988;3:103-11.
44. Pavik K, Piper PJ, Smedegard G. Anaphylatoxin-induced shock and two patterns of anaphylactic shock: hemodynamics and mediators. *Acta Physiol Scand.* 1979;105:393-403.
 45. Lundberg C, Marceau F, Hugi TE. C5a-induced hemodynamic and hematologic changes in the rabbit. Role of cyclooxygenase products and polymorphonuclear leukocytes. *Am J Pathol.* 1987;128:471-83.
 46. Gallin JL. Degranulating stimuli decrease the negative surface charge and increase the adhesiveness of human neutrophils. *J Clin Invest.* 1980;65:298-306.
 47. Frank MM. Complement in the pathophysiology of human disease. *N Engl J Med.* 1987;316:1525-30.
 48. Ford-Hutchinson AW, Bray MA, Dolg MV, Shipley ME, Smith MJ. Leukotriene B₄, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. *Nature.* 1980;286:264-5.
 49. Martin TR, Raugi G, Merritt TL, Henderson WR Jr. Relative contribution of leukotriene B₄ to the neutrophil chemotactic activity produced by the resident human alveolar macrophage. *J Clin Invest.* 1987;80:1114-24.
 50. Pober JS, Lefler LA, Stolphen AH, Brock TA, Springer TA, Fiers W, et al. Activation of cultured human endothelial cells by recombinant lymphotoxin: comparison with tumor necrosis factor and interleukin 1 species. *J Immunol.* 1987;138:3319-24.
 51. Hefner JE, Sahn SA, Repley JE. The role of platelets in the adult respiratory distress syndrome. Culprits or bystanders? *Am Rev Respir Dis.* 1987;135:482-92.
 52. Wahl SM, Hunt DA, Wakefield LM, McCartney-Francis N, Wahl LM, Roberts AB, et al. Transforming growth factor beta induces monocyte chemotaxis and growth factor production. *Proc Natl Acad Sci USA.* 1987;84:5788-92.
 53. Cotran RS, Pober JS. Cytokine-endothelial interactions in inflammation, immunity, and vascular injury. *Journal of the American Society of Nephrology.* 1990;1:225-35.
 54. Mason JW, Kleesberg U, Dolan P, Colman RW. Plasma kallikrein and Hageman factor in Gram-negative bacteremia. *Ann Intern Med.* 1970;75:545-51.
 55. Katori M, Majima M, Odol-Adome R, Sunahara N, Uchida Y. Evidence for the involvement of a plasma kallikrein-kinin system in the immediate hypotension by endotoxin in anesthetized rats. *Br J Pharmacol.* 1989;98:1383-91.
 56. Aasen AO, Smith-Erichsen N, Amundsen E. Plasma kallikrein-kinin system in septicemia. *Arch Surg.* 1983;118:343-6.
 57. Eamon CT. The regulation of natural anticoagulant pathways. *Science.* 1987;235:1348-52.
 58. Bone RC, Francis PB, Pierce AK. Intravascular coagulation associated with the adult respiratory distress syndrome. *Am J Med.* 1976;61:585-9.
 59. Nuijens JH, Eerenberg-Belmer AJ, Huijbregts CC, Schreuder WO, Felt-Berema RJ, Abbink JJ, et al. Proteolytic inactivation of plasma C1-inhibitor in sepsis. *J Clin Invest.* 1989;84:443-50.
 60. Parrillo JE. Septic shock: clinical manifestations, pathogenesis, hemodynamics, and management in a critical care unit. In: Shoemaker WC, Ayres S, Grenvik A, et al., eds. *Textbook of Critical Care Medicine*. 2d ed. Philadelphia: W. B. Saunders; 1989:111-25.
 61. Reilly JM, Cummin RE, Burch-Whitman C, Parker MM, Shelhamer JH, Parrillo JE. A circulating myocardial depressant substance is associated with cardiac dysfunction and peripheral hypoperfusion (lactic acidemia) in patients with septic shock. *Chest.* 1989;95:1072-80.
 62. Suffredini AF, Fromm RE, Parker MM, Brenner M, Kovacs JA, Wesley RA, et al. The cardiovascular response of normal humans to the administration of endotoxin. *N Engl J Med.* 1989;321:280-7.
 63. Benoit F, Blaine J, Lhoste F, Manlikian B, Kerdellue B, Raphin M. Naloxone therapy of human septic shock. *Crit Care Med.* 1985;13:972-5.
 64. Hughes GS Jr. Plasma beta-endorphin-like-immunoreactivity levels and hemodynamics in patients with septic shock. *Res Commun Chem Pathol Pharmacol.* 1985;48:121-31.
 65. Rinaldo JE, Gorry M, Strieter R, Cowan H, Abdolrasulnia R, Shepherd V. Effect of endotoxin-induced cell injury on 70-kD heat shock proteins in bovine lung endothelial cells. *Am J Respir Cell Mol Biol.* 1990;3:207-16.
 66. Elias JA, Freundlich B, Kern JA, Rosenbloom J. Cytokine networks in the regulation of inflammation and fibrosis in the lung. *Chest.* 1990;97:1439-45.
 67. Waage A, Espevik T, Lamvik J. Detection of tumour necrosis-like cytotoxicity in serum from patients with septicaemia but not from untreated cancer patients. *Scand J Immunol.* 1986;24:739-43.
 68. Waage A, Halstensen A, Espevik T. Association between tumour necrosis factor in serum and fatal outcome in patients with meningococcal disease. *Lancet.* 1987;1:355-7.
 69. Girardin E, Grau GE, Dayer JM, Roux-Lombard P, Lambert PH. Tumor necrosis factor and interleukin-1 in the serum of children with severe infectious purpura. *N Engl J Med.* 1988;319:397-400.
 70. Debets JM, Kampmeijer R, van der Linden MP, Boorman WA, van der Linden CJ. Plasma tumor necrosis factor and mortality in critically ill septic patients. *Crit Care Med.* 1989;17:489-94.
 71. Grau GE, Taylor TE, Molyneux ME, Wirlima JJ, Vassalli P, Hommel M, et al. Tumor necrosis factor and disease severity in children with falciparum malaria. *N Engl J Med.* 1989;320:1556-91.
 72. de Groote MA, Martin MA, Densen P, Pfister MA, Wenzel PP. Plasma tumor necrosis factor levels in patients with presumed sepsis. *JAMA.* 1989;262:249-51.
 73. Damas P, Reuter A, Gysen P, Demonty J, Lamy M, Franchimont P. Tumor necrosis factor and interleukin-1 serum levels during severe sepsis in humans. *Crit Care Med.* 1989;17:975-8.
 74. Waage A, Brandtzaeg P, Halstensen A, Kierulf P, Espevik T. The complex pattern of cytokines in serum from patients with meningococcal septic shock. Association between interleukin 6, interleukin 1, and fatal outcome. *J Exp Med.* 1989;169:333-8.
 75. Marks JD, Marks CB, Luce JM, Montgomery AB, Turner J, Metz CA, et al. Plasma tumor necrosis factor in patients with septic shock. *Am Rev Respir Dis.* 1990;141:94-7.
 76. Calandra T, Baumgartner JD, Grau GE, Wu MM, Lambert PH, Schellekens J, et al. Prognostic value of tumor necrosis factor/cachectin, interleukin-1, interferon-alpha, and interferon-gamma in the serum of patients with septic shock. *J Infect Dis.* 1990;161:982-7.
 77. Hesse DG, Tracey KJ, Fong Y, Manogue KR, Palladino MA Jr, Cerami A, et al. Cytokine appearance in human endotoxemia and primate bacteremia. *Surg Gynecol Obstet.* 1988;166:147-53.
 78. Michie HR, Manogue KR, Spriggs DR, Revhaug A, O'Dwyer S, Dinarello CA, et al. Detection of circulating tumor necrosis factor after endotoxin administration. *N Engl J Med.* 1988;318:1481-6.
 79. Born PJ, Patmen N, Engelen JJ, Boorman WA. Spontaneous and stimulated release of tumor necrosis factor-alpha (TNF) from blood monocytes of miners with coal workers' pneumoconiosis. *Am Rev Respir Dis.* 1988;138:1589-94.
 80. Beutler B, Milsark IW, Cerami AC. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. *Science.* 1985;229:869-71.
 81. Tracey KJ, Fong Y, Hesse DG, Manogue KR, Lee AT, Kuo GC, et al. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteremia. *Nature.* 1987;330:662-4.
 82. Opal SM, Cross AS, Kelly NM, Sadoff JC, Bodmer MW, Palardy JE, et al. Efficacy of a monoclonal antibody directed against tumor necrosis factor in protecting neutropenic rats from lethal infection with *Pseudomonas aeruginosa*. *J Infect Dis.* 1990;161:1148-52.
 83. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci USA.* 1975;72:3666-70.
 84. Aderka D, Fisher S, Levo Y, Holtmann H, Hahn T, Wallach D. Cachectin/tumour-necrosis-factor production by cancer patients [Letter]. *Lancet.* 1985;2:1190.
 85. Balkwill F, Osborne R, Burke F, Naylor S, Talbot D, Durbin H, et al. Evidence for tumour necrosis factor/cachectin production in cancer. *Lancet.* 1987;2:1229-32.
 86. Saxne T, Palladino MA Jr, Helnegard D, et al. Detection of tumor necrosis factor alpha but not tumor necrosis factor beta in rheumatoid arthritis synovial fluid and serum. *Arthritis Rheum.* 1988;31:1041-5.
 87. Maury CP, Teppo AM. Tumor necrosis factor in the serum of patients with systemic lupus erythematosus. *Arthritis Rheum.* 1989;32:146-50.
 88. Lahdevirta J, Maury CP, Teppo AM, Reppo H. Elevated levels of circulating cachectin/tumor necrosis factor in patients with acquired immunodeficiency syndrome. *Am J Med.* 1988;85:289-91.
 89. Scuderi P, Sterling KE, Lam KS, Finley PR, Ryan KJ, Ray CG, et al. Raised serum levels of tumour necrosis factor in parasitic infections. *Lancet.* 1986;2:1364-5.
 90. Pisa P, Genzani M, Soder O, Ottenhoff T, Hansson M, Klesling R. Serum tumor necrosis factor levels and disease dissemination in leprosy and leishmaniasis. *J Infect Dis.* 1990;161:988-91.
 91. Bird GL, Sherron N, Goka AK, Alexander GJ, Williams RS. Increased plasma tumor necrosis factor in severe alcoholic hepatitis. *Ann Intern Med.* 1990;112:917-20.
 92. Levine B, Kalman J, Mayer L, Fillet HM, Packer M. Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. *N Engl J Med.* 1990;323:236-41.
 93. Merrill GA, Anderson JH Jr. Involvement of endogenous opiates in glucose-stimulated hyperinsulinism of canine endotoxin shock. Inhibition by naloxone. *Diabetes.* 1987;36:585-91.
 94. Zuckerman SH, Bendele AM. Regulation of serum tumor necrosis factor in glucocorticoid-sensitive and -resistant rodent endotoxin shock models. *Infect Immun.* 1989;57:3009-13.
 95. Brouckaert PG, Everaerd B, Libert C, Takahashi N, Fiers W. Species specificity and involvement of other cytokines in endo-

- toxic shock action of recombinant tumour necrosis factor in mice. Agents Actions. 1989;26:196-8.
96. Ranges GE, Zlotnik A, Espevik T, Dinarello CA, Cerami A, Palladino MA Jr. Tumor necrosis factor-alpha/cachectin is a growth factor for thymocytes. Synergistic interactions with other cytokines. J Exp Med. 1988;167:1472-8.
97. Silva AT, Appelbom BJ, Burman WA, Bayston KF, Cohen J. Monoclonal antibody to endotoxin core protects mice from *Escherichia coli* sepsis by a mechanism independent of tumor necrosis factor and interleukin-6. J Infect Dis. 1990;162:454-9.
98. Smith JG, Magee DM, Williams DM, Graybill JR. Tumor necrosis factor-alpha plays a role in host defense against *Histoplasma capsulatum*. J Infect Dis. 1990;162:1349-53.
99. Kurt-Jones EA, Fiers W, Pober JS. Membrane interleukin 1 induction on human endothelial cells and dermal fibroblasts. J Immunol. 1987;139:2317-24.
100. Bessin P, Bonnet J, Apffel P, Soulard C, Desgroux L, Petais I, et al. Acute circulatory collapse caused by platelet-activating factor (PAF-acether) in dogs. Eur J Pharmacol. 1983;86:403-13.
101. Lefer AM, Muller HF, Smith JB. Pathophysiological mechanisms of sudden death induced by platelet activating factor. Br J Pharmacol. 1984;83:125-30.
102. Ligumsky M, Simon PL, Karmeli F, Rachmilewitz D. Role of interleukin 1 in inflammatory bowel disease—enhanced production during active disease. Gut. 1990;31:686-9.
103. Prieur AM, Kaufmann MT, Griscelli C, Dayer JM. Specific interleukin-1 inhibitor in serum and urine of children with systemic juvenile chronic arthritis. Lancet. 1987;2:1240-2.
104. Eastgate JA, Simons JA, Wood NC, Grindinton FM, di Giovine FS, Duff GW. Correlation of plasma interleukin 1 levels with disease activity in rheumatoid arthritis. Lancet. 1988;2:706-9.
105. Eden E, Turino GM. Interleukin 1 secretion from human alveolar macrophages in lung disease. J Clin Immunol. 1986;6:326-33.
106. Smith LJ, Rubin AH, Patterson R. Mechanism of platelet activating factor-induced bronchoconstriction in humans. Am Rev Respir Dis. 1988;137:1015-9.
107. Egid J, Robles A, Ortiz A, Ramirez F, Gonzalez E, Mampaso F, et al. Role of platelet-activating factor in adriamycin-induced nephropathy in rats. Eur J Pharmacol. 1987;138:119-23.
108. Balk RA, Jacobs RF, Tryba F, Townsend JW, Walls RC, Bone RC. Effects of ibuprofen on neutrophil function and acute lung injury in canine endotoxin shock. Crit Care Med. 1988;16:1121-7.
109. de Nucci G, Thomas R, D'Orleans-Juste P, Antunes E, Walder C, Warner TD, et al. Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. Proc Natl Acad Sci USA. 1988;85:9797-800.
110. Beutler B, Kronchin N, Millsark IW, Luedke C, Cerami A. Control of cachectin (tumor necrosis factor) synthesis: mechanisms of endotoxin resistance. Science. 1986;232:977-80.
111. Henderson WR, Klebanoff SJ. Leukotriene production and inactivation by normal, chronic granulomatous disease and myeloperoxidase-deficient neutrophils. J Biol Chem. 1983;258:13522-7.
112. Beutler LD, Layman NK, Cain RL, Riedl PE, Mobler KM, Bobbitt JL, et al. Interleukin 1-induced pathophysiology: induction of cytokines, development of histopathologic changes, and immunopharmacologic intervention. Clin Immunol Immunopathol. 1989;53:400-21.
113. Dinarello CA. Interleukin-1. Rev Infect Dis. 1984;6:55-95.
114. Zockerman JH, Shelhaas J, Butler LD. Differential regulation of lipopolysaccharide-induced interleukin 1 and tumor necrosis factor synthesis: effects of endogenous glucocorticoids and the role of the pituitary-adrenal axis. Eur J Immunol. 1989;19:301-5.
115. Lefer AM. Induction of tissue injury and altered cardiovascular performance by platelet-activating factor: relevance to multiple systems organ failure. Crit Care Clin. 1989;5:331-51.
116. Inarrea P, Gomez-Cambronero J, Pascual J, Ponte MC, Hernandez L, Sanchez-Crespo M. Synthesis of PAF-acether and blood volume changes in gram-negative sepsis. Immunopharmacology. 1985;9:45-52.
117. Oda M, Satouchi K, Ikeda I, Sakakura M, Yasunaga K, Saito K. The presence of platelet-activating factor associated with eosinophil and/or neutrophil accumulations in the pleural fluids. Am Rev Respir Dis. 1990;141:1469-73.
118. Rothstein JL, Schreiber H. Synergy between tumor necrosis factor and bacterial products causes hemorrhagic necrosis and lethal shock in normal mice. Proc Natl Acad Sci USA. 1988;85:607-11.
119. Kunkel SL, Spengler M, May MA, Spengler R, Larrick J, Resnick D. Prostaglandin E2 regulates macrophage-derived tumor necrosis factor gene expression. J Biol Chem. 1988;263:5380-4.
120. Myers AK, Robey JW, Price RM. Relationships between tumor necrosis factor, eicosanoids and platelet-activating factor as mediators of endotoxin-induced shock in mice. Br J Pharmacol. 1990;99:499-502.
121. Sun XM, Hsueh W, Torre-Amione G. Effects of in vivo "priming" on endotoxin-induced hypotension and tissue injury. The role of PAF and tumor necrosis factor. Am J Pathol. 1990;136:949-56.
122. Mehta JL, Lawson DL, Nichols WW. Attenuated coronary relaxation after reperfusion: effects of superoxide dismutase and TxA2 inhibitor U 63557A. Am J Physiol. 1989;257(4 Pt 2):H1240-6.
123. Marsden PA, Goligorsky MS, Brenner BM. Endothelial cell biology in relation to current concepts of vessel wall structure and function. Journal of the American Society of Nephrology. 1991;1:931-48.
124. Kunkel SL, Strieter RM. Cytokine networking in lung inflammation. Hosp Pract. 1990;25(10):63-76.
125. Warren RS, Starnes HF Jr, Gabrilove JL, Oettingen HF, Brennan MF. The acute metabolic effects of tumor necrosis factor administration in humans. Arch Surg. 1987;122:1396-400.
126. Clark RA, Klebanoff SJ. Chemotactic factor inactivation by the myeloperoxidase-hydrogen peroxide-halide system. J Clin Invest. 1979;64:913-20.
127. Clark RA, Borregaard N. Neutrophils autoinactivate secretory products by myeloperoxidase-catalyzed oxidation. Blood. 1985;65:375-81.
128. Warner SJ, Auger KR, Libby P. Interleukin 1 induces interleukin 1. II. Recombinant human interleukin 1 induces interleukin 1 production by adult human vascular endothelial cells. J Immunol. 1987;139:1911-7.
129. Holt PG. Down-regulation of the immune responses in the lower respiratory tract: the role of the alveolar macrophages. Clin Exp Immunol. 1986;63:261-70.
130. Utsuni K, Takai Y, Tada T, Ohzaki S, Fujiwara H, Hamaoka T. Enhanced production of IL-6 in tumor-bearing mice and determination of cells responsible for its augmented production. J Immunol. 1990;145:397-403.
131. Piper PJ, Letts LG, Galton SA. Generation of a leukotriene-like substance from porcine vascular and other tissues. Prostaglandins. 1983;25:591-9.
132. Bernstein A, Sibbald WJ. Acute lung injury in septic shock. Crit Care Clin. 1989;5:49-79.
133. Snapper JR, Hinson JM Jr, Hutchinson AA, Lefferts PL, Ogletree ML, Brigham KL. Effect of platelet depletion on the unanesthetized sheep's pulmonary response to endotoxemia. J Clin Invest. 1984;74:1782-91.
134. Millar AB, Foley NM, Singer M, Johnson NM, Meager A, Rook GA. Tumour necrosis factor in bronchopulmonary secretions of patients with adult respiratory distress syndrome. Lancet. 1989;2:712-4.
135. Beutler BA, Millsark IW, Cerami A. Cachectin/tumor necrosis factor: production, distribution, and metabolic fate in vivo. J Immunol. 1985;135:3972-7.
136. Palladino MA Jr, Shalaby MR, Kramer SM, Ferradolo BL, Baughman RA, Deleo AB, et al. Characterization of the antitumor activities of human tumor necrosis factor-alpha and the comparison with other cytokines: induction of tumor-specific immunity. J Immunol. 1987;138:4023-32.
137. Wallace JL, Steel G, Whittle BJ, Lagente V, Vargaflik B. Evidence for platelet-activating factor as a mediator of endotoxin-induced gastrointestinal damage in the rat. Effects of three platelet-activating factor antagonists. Gastroenterology. 1987;93:765-73.
138. Kon V, Yoshioka T, Fogo A, Ichikawa I. Glomerular actions of endothelin in vivo. J Clin Invest. 1989;83:1762-7.
139. Jacobs RF, Kiel DP, Balk RA. Alveolar macrophage function in a canine model of endotoxin-induced lung injury. Am Rev Respir Dis. 1986;134:745-51.
140. Schwartz DB, Bone RC, Balk RA, Seldon JP. Hepatic dysfunction in the adult respiratory distress syndrome. Chest. 1989;95:871-5.
141. Okusawa S, Gelfand JA, Ikejima T, Connolly RJ, Dinarello CA. Interleukin 1 induces a shock-like state in rabbits. Synergism with tumor necrosis factor and the effect of cyclooxygenase inhibition. J Clin Invest. 1988;81:1162-72.